Supplementary Information

Legends to Supporting Figures

FIGURE S1. SDS-PAGE analysis of recombinantly expressed and purified desmin mutants. 10 µg protein was used to verify the purity. The gel was stained with Coomassie Blue R250. The purity of all desmin mutants was higher than 95%.

FIGURE S2. Controls for PALM experiments. After transfection of SW-13 cells with the expression plasmid for desmin-N116S-mEosFP*thermo*PALM images were generated in the red (**A**) and in the green channel (**B**) to demonstrate that mEosFP*thermo* is converted completely under the used conditions. To show that mIrisGFP1cannot be photoconverted to a red emitting form, cells were separately transfected with an expression plasmid for desmin-mIrisGFP1. PALM images are recorded in the red (**C**) and in the green channel (**D**). To exclude damage of the transfected cells induced by high intensity light during conversion and acquisition DIC images are taken pre (**E**) and post PALM (**F**). Indicated by an unimpaired cell shape adverse effects of the used illumination protocol can be excluded.

FIGURE S3. Immunoblot analysis for the detection of exogenous and endogenous desmin. The H9c2- **(A1)**; C2C12- **(A2)** and HL-1-cells **(A3)** were transfected with the different desmin-eYFP constructs. 24 h after transfection the cell lysates were analyzed by SDS-PAGE and immunoblot analysis using desmin antibodies. Of note, the untransfected cells express

endogenous desmin and contribute to the endogenous signal in the immunoblot analysis. Therefore, the transfection efficiency of each cell line was determined by fluorescence activated cell sorting (FACS) to calculate a corrected ratio of exogenous and endogenous desmin **(B)**.

Figure S1.

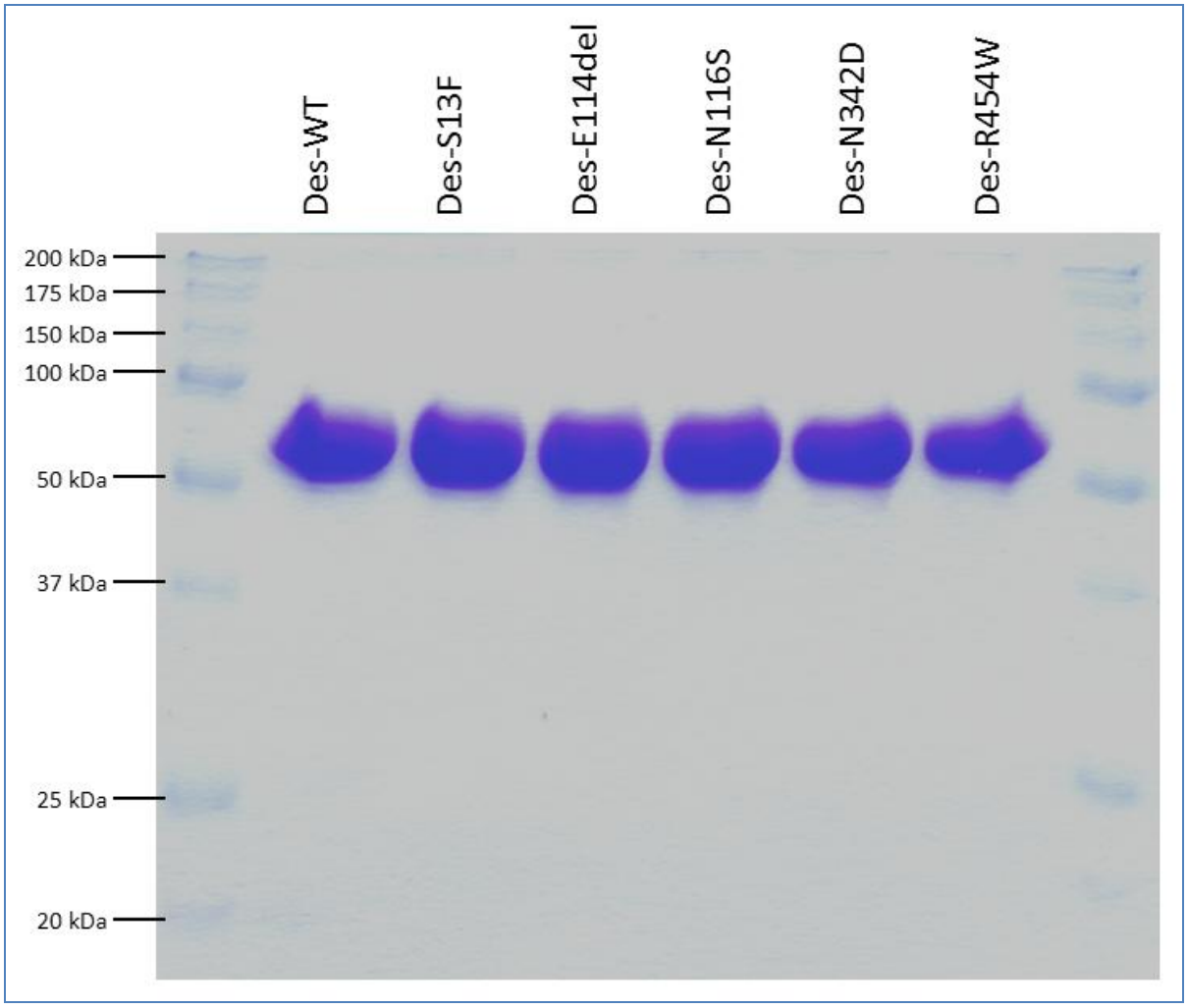

Des-WT
Des-S13F
Des-E114del
Des-N116S
Des-N342D
Des-R454W
200 kDa
175 kDa
150 kDa
100 kDa
50 kDa
37 kDa
25 kDa
20 kDa

Figure S2.

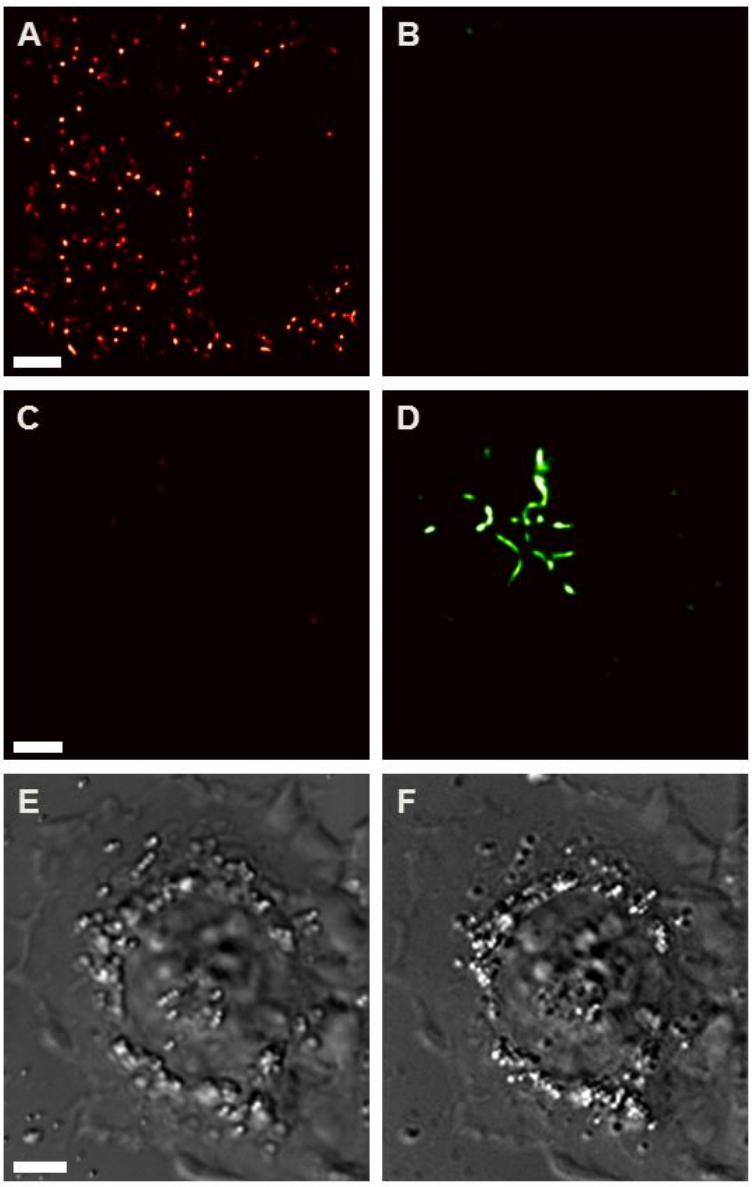
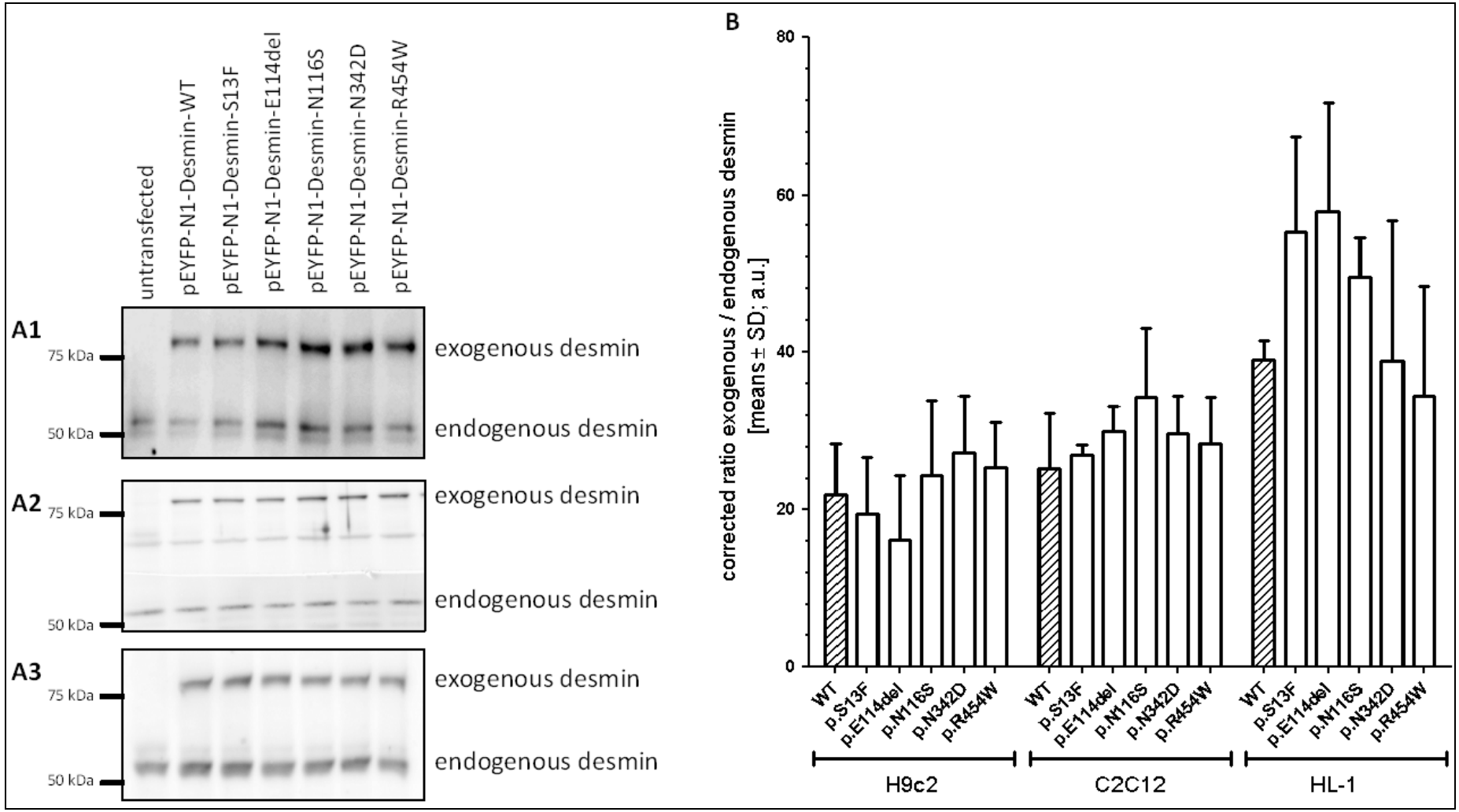


Figure S3


untransfected
pEYFP-N1-Desmin-WT
pEYFP-N1-Desmin-S13F
pEYFP-N1-Desmin-E114del
pEYFP-N1-Desmin-N116S
pEYFP-N1-Desmin-N342D
pEYFP-N1-Desmin-R454W
A1
75 kDa
50 kDa
exogenous desmin
endogenous desmin
A2
75 kDa
50 kDa
exogenous desmin
endogenous desmin
A3
75 kDa
50 kDa
exogenous desmin
endogenous desmin
B
corrected ratio exogenous / endogenous desmin
[means ± SD; a.u.]
0
20
40
60
80
WT
p.S13F
p.E114del
p.N116S
p.N342D
p.R454W
H9c2
C2C12
HL-1

Vector constructs

Various desmin constructs were generated by cloning full-length cDNA of human desmin into the vectors pEYFP-N1, pECFP-N1 (Clontech Laboratories, CA, USA) and pET100D-TOPO (Invitrogen, Carlsbad, USA). The mutations were introduced by site-directed mutagenesis (QuickChange Lightning, Agilent Technologies, Santa Clara, USA) using appropriate oligonucleotides (Supplementary Information, Table SI) according to the manufacturer's instructions. To generate expression vectors for untagged desmin-constructs, the eYFP-tag was removed and afterwards replaced by hybridized oligonucleotides containing a stop-codon. For PALM-analysis, eYFP- and eCFP-tags were replaced by mEosFP*thermo* (mEosFP A69V) and mIrisGFP1 (mEosFP*thermo* F173S). Each plasmid was controlled by sequencing using the Big Dye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, USA) on an ABI-310 genetic analyzer (Applied Biosystems, Foster City, USA).

Table SI: *Designation of constructs and methods of cloning*

Primers[a]:(1) agactcgaggccgtcaccatgagccaggcctactcgtccagcc; (2) acaggatccccgagcacttcatgctgctgctgtgtgg; (3) agcgcgtgtccttctaccgccgcac; (4) gtgcggcggtagaaggacacgcgct; (5) aaggtggagctgcagctcaatgaccgcttc; (6) gaagcggtcattgagctgcagctccaccttt; (7) caggagctcagtgaccgcctt; (8) gaagcggtcactgagctcctg; (9) gacgccctgaagggcactgacgattccctg; (10) cagggaatcgtcagtgcccttcagggcgtc; (11) atgatcaagaccatcgagacatgggatggggag; (12) ctccccatcccatgtctcgatggtcttgatcat; (13) ccggatccaccggtcgccaccatgagtgcgattaagccagacatgaagatc; (14) gtcgcggccgctttatcgtctggcattgtcaggcaatcc; (15) caccatgagccaggcctactc; (16) ttagagcacttcatgctgctgct; (17) gagctgcaggagctcgctgaccgcttcgccaa; (18) ttggcgaagcggtcagcgagctcctgcagctc; (19) gtggagctgcaggagctcactgaccgcttc; (20) gaagcggtcagtgagctcctgcagctccac; (21) gttggcgaagcggtcctggagctcctgcagctc; (22) gttggcgaagcggtcctggagctcctgcagctc; (23) gctgcaggagctcgatgaccgcttcgc; (24) gcgaagcggtcatcgagctcctgcagc; (25) ccggtgtaggtaggtagaagcttgtcgacgc; (26) ggccgcgtcgacaagcttctacctacctaca;

Construct No.	Construct designation	Source of cDNA/ method of cloning	Restriction sides	Primers
1	pEYFP-N1-Desmin	PCR of pCMV6-AC-Desmin[b] / TOPO-TA	XhoI, BamHI	1, 2
2	pEYFP-N1-Desmin-S13F	SDM of pEYFP-N1-Desmin	XhoI, BamHI	3, 4
3	pEYFP-N1-Desmin-E114del	SDM of pEYFP-N1-Desmin	XhoI, BamHI	5, 6
4	pEYFP-N1-Desmin-N116S	PCR of pET100D-TOPO-Desmin-N116S, TOPO-TA	XhoI, BamHI	7, 8
5	pEYFP-N1-Desmin-N116A	SDM of pEYFP-N1-Desmin	XhoI, BamHI	17, 18
6	pEYFP-N1-Desmin-N116T	SDM of pEYFP-N1-Desmin	XhoI, BamHI	19, 20
7	pEYFP-N1-Desmin-N116Q	SDM of pEYFP-N1-Desmin	XhoI, BamHI	21, 22
8	pEYFP-N1-Desmin-N116D	SDM of pEYFP-N1-Desmin	XhoI, BamHI	23, 24
9	pEYFP-N1-Desmin-N342D	SDM of pEYFP-N1-Desmin	XhoI, BamHI	9, 10
10	pEYFP-N1-Desmin-R454W	SDM of pEYFP-N1-Desmin	XhoI, BamHI	11, 12
11	pECFP-N1-Desmin	PCR of pCMV6-AC-Desmin[a] / TOPO-TA	XhoI, BamHI	1, 2
12	pECFP-N1-Desmin-S13F	SDM of pECFP-N1-Desmin	XhoI, BamHI	3, 4
13	pECFP-N1-Desmin-E114del	SDM of pECFP-N1-Desmin	XhoI, BamHI	5, 6
14	pECFP-N1-Desmin-N116S	PCR of pET100D-TOPO-Desmin-N116S, TOPO-TA	XhoI, BamHI	7, 8

15	pECFP-N1-Desmin-N342D	SDM of pECFP-N1-Desmin	XhoI, BamHI	9, 10
16	pECFP-N1-Desmin-R454W	SDM of pECFP-N1-Desmin	XhoI, BamHI	11, 12
17	pmEosFPthermo-Desmin	PCR of EosFPthermo[c]/ TOPO-TA / subcloning into pEYFP-N1-Desmin-WT	AgeI, NotI	13, 14
18	pmEosFPthermo-Desmin-S13F	PCR of EosFPthermo[c]/ TOPO-TA / subcloning into pEYFP-N1-Desmin-S13F	AgeI, NotI	13, 14
19	pmEosFPthermo-Desmin-E114del	PCR of EosFPthermo[c]/ TOPO-TA / subcloning into pEYFP-N1-Desmin-E114del	AgeI, NotI	13, 14
20	pmEosFPthermo-Desmin-N116S	PCR of EosFPthermo[c]/ TOPO-TA / subcloning into pEYFP-N1-Desmin-N116S	AgeI, NotI	13, 14
21	pmEosFPthermo-Desmin-N342D	PCR of EosFPthermo[c]/ TOPO-TA / subcloning into pEYFP-N1-Desmin-N342D	AgeI, NotI	13, 14
22	pmEosFPthermo-Desmin-R454W	PCR of EosFPthermo[c]/ TOPO-TA / subcloning into pEYFP-N1-Desmin-R454W	AgeI, NotI	13, 14
23	pmIrisGFP1-Desmin	PCR of mIrisGFP1[c]/ TOPO-TA / subcloning into pEYFP-N1-Desmin-WT	AgeI, NotI	13, 14
24	pmIrisGFP1-Desmin-S13F	PCR of mIrisGFP1[c] / TOPO-TA / subcloning into pEYFP-N1-Desmin-S13F	AgeI, NotI	13, 14
25	pmIrisGFP1-Desmin-E114del	PCR of mIrisGFP1[c] / TOPO-TA / subcloning into pEYFP-N1-Desmin-E114del	AgeI, NotI	13, 14
26	pmIrisGFP1-Desmin-N116S	PCR of mIrisGFP1[c] / TOPO-TA / subcloning into pEYFP-N1-Desmin-N116S	AgeI, NotI	13, 14
27	pmIrisGFP1-Desmin-N342D	PCR of mIrisGFP1[c] / TOPO-TA / subcloning into pEYFP-N1-Desmin-N342D	AgeI, NotI	13, 14
28	pmIrisGFP1-Desmin-R454W	PCR of mIrisGFP1[c] / TOPO-TA / subcloning into pEYFP-N1-Desmin-R454W	AgeI, NotI	13, 14
29	pET100D-TOPO-Desmin	PCR of pCMV6-AC-Desmin, directional TOPO cloning[d]	-	15, 16
30	pET100D-TOPO-Desmin-S13F	SDM of pET100D-TOPO-Desmin	-	3, 4
31	pET100D-TOPO-Desmin-E114del	SDM of pET100D-TOPO-Desmin	-	5, 6
32	pET100D-TOPO-Desmin-N116S	PCR with overlapping primers, directional TOPO cloning[d]	-	7, 8
33	pET100D-TOPO-Desmin-N342D	SDM of pET100D-TOPO-Desmin	-	9, 10
34	pET100D-TOPO-Desmin-R454W	SDM of pET100D-TOPO-Desmin	-	11, 12
35	pDes-WT	Replacement of eYFP (pEYFP-Des-WT) against a stop-codon	AgeI, NotI	25, 26

36	pDes-S13F	Replacement of eYFP (pEYFP-Des-S13F) against a stop-codon	AgeI, NotI	25, 26
37	pDes-E114del	Replacement of eYFP (pEYFP-Des-E114del) against a stop-codon	AgeI, NotI	25, 26
38	pDes-N116S	Replacement of eYFP (pEYFP-Des-N116S) against a stop-codon	AgeI, NotI	25, 26
39	pDes-N342D	Replacement of eYFP (pEYFP-Des-N342D) against a stop-codon	AgeI, NotI	25, 26
40	pDes-R454W	Replacement of eYFP (pEYFP-Des-R454W) against a stop-codon	AgeI, NotI	25, 26

[a] 5‘-3‘ sequence orientation, all primers were synthesized by Microsynth, Balgach, CH

[b] Origene Technologies, Rockville, USA

[c] cDNAs of mEosFP*thermo* and mIrisGFP1 (in pQE30 or pQE32) were kindly provided by the group of Gerd Ulrich Nienhaus.

[d] Described previously by (Klauke et al, 2010).